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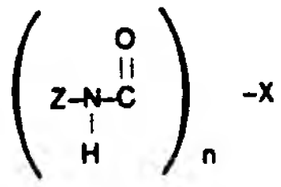
(54) Pharmaceutical composition containing a tetrapyrrole compound as active ingredient and process for the production of the tetrapyrrole compound.

(57) This invention relates to new therapeutic compositions for detection and/or treatment of mammalian tumors which comprises a fluorescent mono- or polyamide of an aminodicarboxylic acid and a tetrapyrrole containing at least one carboxy group of the structure:



wherein Z is the aminodicarboxylic acid residue less the amino group and X is the tetrapyrrole residue less the carboxy group and «n» is an integer from 1 to 4 inclusive, and a pharmaceutical carrier therefor and a process for preparing the active tetrapyrrole compound.

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PHARMACEUTICAL COMPOSITION CONTAINING A TETRAPYRROLE COMPOUND AS ACTIVE INGREDIENT AND PROCESS FOR THE PRODUCTION OF THE TETRAPYRROLE COMPOUND

This invention relates to new therapeutic compositions which are useful in photodiagnosis and phototherapy, especially in the detection and treatment of tumors and cancerous tissues in the human or animal body.

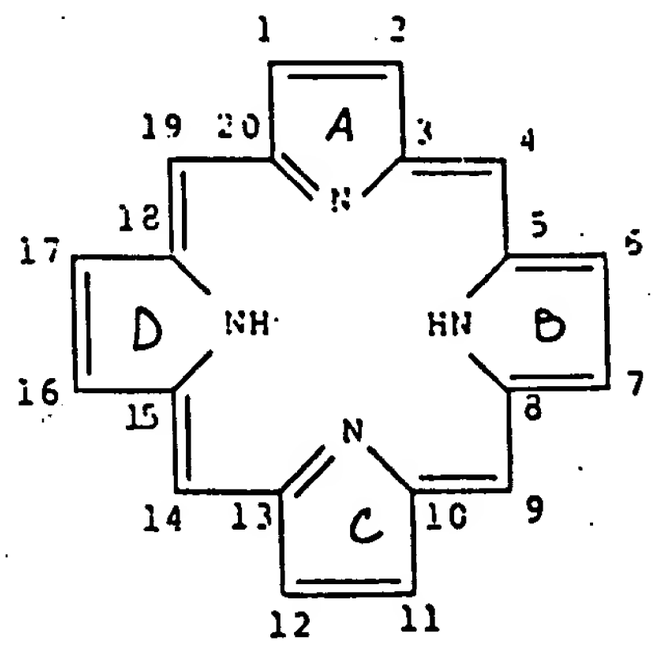
It is known to irradiate tumors and cancerous tissues in the human body with intensive light following administration of a hematoporphyrin derivative in the wavelength range of 626 to 636 nanometers to reduce and, at times, destroy the cancerous cells (see PCT published specification WO 83/00811). It is also known that porphyrins, especially the sodium salt of protoporphyrins, can maintain or promote the normal functions of cells and are useful for preventing the genesis, growth, metastasis , and relapse of malignant tumors. Japanese Published Patent Application No. 125737/76 describes the use of porphyrins as tumor inhibiting agents, exemplifying etioporphyrin, mesoporphyrin, protoporphyrin, deuteroporphyrin, hematoporphyrin, coprophyrin, and uroporphyrin.

In Tetrahedron Letters No. 23, pp. 2017-2020 (1978), there is described an amino monocarboxylic acid adduct of the pigment bonellin obtained by extraction of principally the body wall of the marine echuroid B. viridis. The structure of these adducts is presumed to be an amide formed through either of the free carboxy groups of bonellin and the amino monocarboxylic acid. Hydrolysis of the adduct yielded

a mixture of valine, isoleucine, leucine and allo-isoleucine. No use for these amino acid adducts is described in this reference.

That the tetrapyrroles cause intense photosensitivity in animals is well-known and has been documented in numerous articles in literature, e.g., J. Intr. Sci. Vitaminol, 27, 521-527 (1981); Agric. Biol. Chem., 46(9), 2183-2193 (1982); Chem. Abst. 98, 276 (1983) and 88, 69764m (1928).

The therapeutic agents contemplated by this invention are cyclic and acyclic tetrapyrroles derived by various procedures from naturally-occurring tetrapyrroles. The cyclic tetrapyrroles have as their common parent tetrapyrrole, uroporphyrinogen, and possess the following ring structure:



in which the positions in the molecule are numbered 1-20, and the rings identified by letters A, B, C and D, and also include perhydro-, e.g., dihydro- and tetrahydro-, derivatives of the said ring structure, e.g., compounds in which one or more double bonds are absent. There are present in the ring system four pyrrole rings joined through the alpha positions of the respective pyrrole rings by a methine group, i.e., -CH=. The compounds of the present invention are designated as derivatives of the tetrapyrroles for convenience in the disclosure and the appended claims and it will be understood that the term "tetrapyrrole" will designate compounds of the characteristic ring structure designated hereinbefore as well as the corresponding perhydro derivatives, and the corresponding non-cyclic pyrroles, i.e., the linear tetrapyrroles, commonly known as the bile pigments.

The tetrapyrroles employed in the present invention are all derived by various means and various alteration procedures from natural tetrapyrroles. The naturally occurring tetrapyrroles have as their common ancestor uroporphyrinogen III, a hexahydroporphyrin reduced at the bridge positions. For example, synthetic or biosynthetic derivatives or products of protoporphyrins IX or protoporphyrinogen IX are well-known in the art (see, for example, Porphyrins and Metalloporphyrins, K. Smith Elsivier; The Porphyrins (Vols. 1-7) D. Dolphin, Academic Press; and Biosynthetic Pathways, Vol. III, Chapter by B. Burnham, editor D.M. Greenberg, Academic Press).

The non-cyclic tetrapyrroles are commonly known as bile pigments and include, for example, bilirubin and biliverdin. These tetrapyrroles are also derived from protoporphyrin, e.g., as metabolic products in animals.

A further characteristic of the present new therapeutic composition is the presence of at least one amide linkage in a substituent at any of the numbered positions of the ring structure. These are present in the instant new compounds together with other substituents as defined hereinafter.

Thus, the present invention contemplates the therapeutic compositions comprising of amino acid or peptide derivatives of compounds which contain chromosphore of porphyrins, chlorins or bacteriochlorins, as well as related porphyrin compounds. The peptide linkage involves a carboxy group of the chromophore-bearing compound and the amino group of the specified amino acid. The present new compounds embrace, inter alia, derivatives of the tetrapyrroles which contain a free carboxy group. These derivatives include the major classes of tetrapyrroles: carboxy-containing porphyrins, chlorins, and bacteriochlorins, which are well-known to those skilled in this art.

The amino acid employed in the present invention to form the aforesaid peptide linkage are amino-dicarboxylic acids in which the amino group, of course, is located

on a carbon atom of the dicarboxylic acid. The specific position of the amino group in the carbon atom chain is not critical, the only requirement being that the amino group be available to form the requisite peptide linkage with the carboxyl group of the selected porphyrin. Thus, a variety of amino dicarboxylic acids are useful in the composition of the present invention, including α-aminosuccinic (aspartic), α-aminoglutaric (glutamic), beta-aminoglutaric, beta-aminosebacic, 2,6-piperidinedicarboxylic, 2,5-pyrrole-dicarboxylic, 2-carboxypyrrole-5-acetic, 2-carboxy-piperidine-6-propionic, α-aminoadipic, α-aminoazelaic, and similar such acids. These amino acids may be substituted with angular alkyl groups such as methyl and ethyl groups, as well as other groups which do not adversely affect the capability of the amino group to form the peptide linkage, e.g., alkoxy groups or acyloxy groups, and may also include additional amino groups. The preferred amino acids are the naturally occurring α-amino acids, glutamic and aspartic acids, whch are readily available and, up to the present, have provided the best results.

Exemplary compounds of the tetrapyrrole classes are illustrated in Table I in which the numbered positions of the tetrapyrrole ring structure are used to designate the position of the indicated substituent. The absence of double bonds in the ring system is designated under "dihydro" with each set of numbers (ring position) indicating the absence of a double bond between the designated positions.

TABLE I

	Ring Position									
	A			B		C			D	
PORPHYRIN	1	2	6	7	11	12	14	16	17	Dihydro
Coproporphyrin III	Me	Pr	Me	Pr	Me	Pr	H	Pr	Me	---
Deuteroporphyrin IX	Me	H	Me	H	Me	Pr	H	Pr	Me	---
Hematoporphyrin IX	Me	Me | -CH | OH	Me	Me | -CH | OH	Me	Pr	H	Pr	Me	---
Protoporphyrin IX	Me	V	Me	V	Me	Pr	H	Pr	Me	---
Photoprotoporphyrin IX	Me	V	{ -Me { -OH	=CHCH$_2$	Me	Pr	H	Pr	Me	6,7
Mesoporphryin IX	Me	Et	Me	Et	Me	Pr	H	Pr	Me	---
Pyropheophorbide a	Me	V	Me	Et	Me	C(=O)—CH$_2$		{ Pr { H	{ Me { H	16,17
Transmesochlorin IX	{ H { Me	{ H { Et	Me	Et	Me	Pr	H	Pr	Me	1,2
Transmesochlorin IX	Me	Et	{ H { Me	{ H { Et	Me	Pr	H	Pr	Me	6,7
Pheophorbide a	Me	V	Me	Et	Me	C(=O)—CH(CO_2Me)		{ Pr { H	{ Me { H	16,17

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TABLE I - Cont'd.

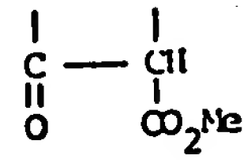
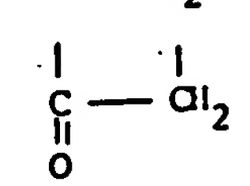
PORPHYRIN	Ring Position A 1	A 2	B 6	B 7	C 11	C 12	C 14	D 16	D 17	Dihydro
Chlorin e_4	Me	V	Me	Et	Me	CO_2H	Me	H / Pr	H / Me	16,17
Chlorin e_6	Me	V	Me	Et	Me	CO_2H	Ac	H / Pr	H / Me	16,17
Mesochlorin e_4	Me	Et	Me	Et	Me	CO_2H	Me	H / Pr	H / Me	16,17
Isochlorin e_4	Me	V	Me	Et	Me	H	Ac	H / Pr	H / Me	16,17
Mesoisochlorin e_4	Me	Et	Me	Et	Me	H	Ac	H / Pr	H / Me	16,17
Mesochlorin e_6	Me	Et	Me	Et	Me	CO_2H	Ac	H / Pr	H / Me	16,17
Bacteriopheophorbide a	Me	ACL	H / Me	H / Et	Me			H / Pr	H / Me	6,7 16,17
Pyrobacteriopheophorbide a	Me	ACL	H / Me	H / Et	Me			H / Pr	H / Me	6,7 16,17
Bacteriochlorin e_6	Me	ACL	H / Me	H / Et	Me	CO_2H	Ac	H / Pr	H / Me	6,7 16,17

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TABLE I - Cont'd.

PORPHYRIN	Ring Position A 1	A 2	B 6	B 7	C 11	C 12	D 14	D 16	D 17	Dihydro
Bacteriochlorin e_4	Me	ACL	H Me	H Et	Me	CO_2H	Me	H Pr	H Me	6,7 16,17
Bacterioisochlorin e_4	Me	ACL	H Me	H Et	Me	H	Ac	H Pr	H Me	6,7 16,17

Notes:

Me:	$-CH_3$ (Methyl group)
Pr:	$-CH_2CH_2COOH$ (Propionic acid group)
V:	$-CH=CH_2$ (Vinyl group)
Et:	$-CH_2CH_3$ (Ethyl group)
Ac:	$-CH_2COOH$ (Acetic acid group)
ACL:	CH_3-CO- (Acetyl group)

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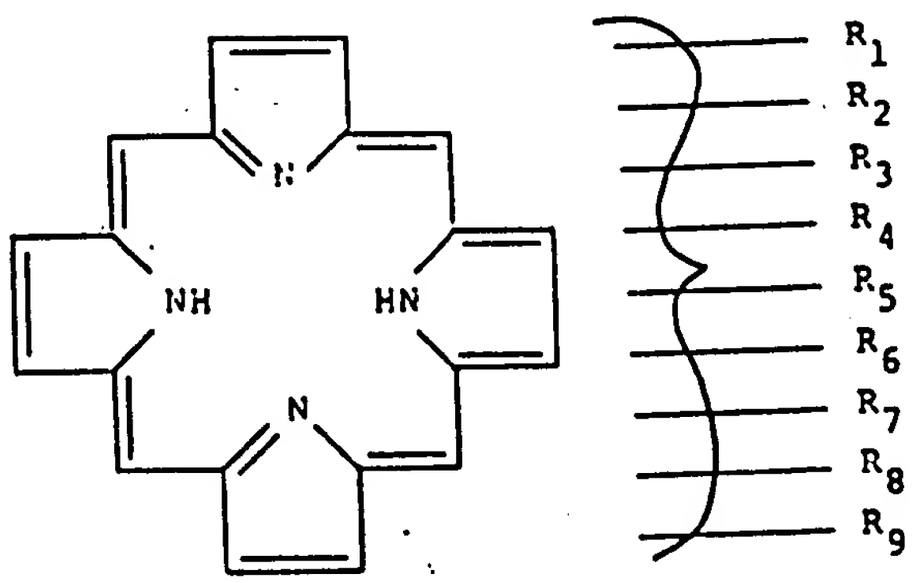
The present new therapeutic composition is comprised of mono- or polyamides of an aminodicarboxylic and a tetrapyrrole containing at least one carboxyl group of the structure

$$\left(Z - \underset{\underset{H}{|}}{N} - \overset{\overset{O}{\|}}{C} \right)_n - X$$

wherein Z is the aminodicarboxylic acid residue less the amino group and X is the tetrapyrrole residue less the carboxy group and "n" is an integer from 1 to 4 inclusive.

The particularly preferred compounds are fluorescent mono- or polyamides of an aminodicarboxylic acid and a tetrapyrrole compound of the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-OH\\-CH_3;\end{cases}$

R_2 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CHCH_3}$, acetyl, $\begin{cases}-H\\-ethyl,\end{cases}$ $-\overset{\overset{H}{|}}{C}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-CH_3\\-OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CHCH_3}$, $CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H\\-ethyl;\end{cases}$

R_5 is methyl;

R_6 is H, $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$ or CO_2H;

R_7 is $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$, or $\begin{cases}-CH_2CH_2CO_2H\\-H;\end{cases}$

R_8 is methyl or $\begin{cases}-CH_3\\-H\end{cases}$

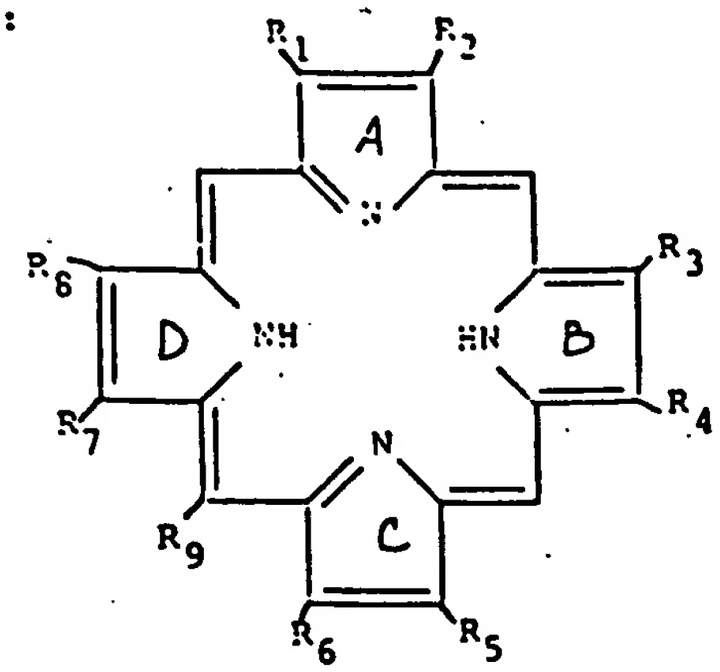
R_9 is H, COOH, CH_2COOH or methyl;

provided that when R_1, R_2, R_3, R_4, R_7 and R_8 represent two substituents or are divalent and attached to the same carbon the respective pyrrole ring to which attached is a dihydropyrrole;

R is lower alkyl or benzyl; R_6 and R_9, taken together are $-\underset{\underset{}{|}}{C}=O$ $-CH_2$ or $-\underset{\underset{}{|}}{C}=O$ $-CHCO_2CH_3$

with the proviso that at least one of R_1-R_9 includes a free carboxyl group; and salts thereof.

The especially preferred therapeutic compositions of the invention are comprised of amides which are derived from tetrapyrroles of the formula:



or the corresponding di- or tetrahydrotetrapyrroles and salts thereof , wherein R_1 - R_9 are as previously defined.

Particularly preferred therapeutic agents of this inventi include the following compounds:

Chlorin Derivatives

Mono and diaspartyl trans-mesochlorin IX
Mono and diglutamyl trans-mesochlorin IX
Mono, di and triaspartyl chlorin e_6
Mono, di and triaspartyl mesochlorin e_6
Mono, di and triglutamyl chlorin e_6
Mono, di and triglutamyl mesochlorin e_6
Mono and diaspartyl chlorin e_4
Mono and diaspartyl mesochlorin e_4
Mono and diaspartyl isochlorin e_4
Mono and diaspartyl mesochlorin e_4
Mono and diglutamyl chlorin e_4
Mono and diglutamyl mesochlorin e_4
Mono and diglutamyl isochlorin e_4
Mono and diglutamyl mesoisochlorin e_4
Monoaspartyl pyropheophorbide a
Monoglutamylpyropheophorbide a
Monoaspartylpheophorbide a
Monoglutamylpheophorbide a
Mono and diaspartylphotoprotoporphyrin IX
Mono and diglutamylphotoprotoporphyrin IX
Mono and di-L-alpha-aminoadipyl trans-mesochlorin IX

Porphyrins Derivatives

Mono and diaspartylmesoporphyrin IX
Mono and diglutamylmesoporphyrin IX
Mono and diaspartylprotoporphyrin IX
Mono and diglutamyl protoporphyrin IX
Mono and diaspartyldeuteroporphyrin IX
Mono and diglutamyldeuteroporphyrin IX
Mono, di, tri and tetraaspartylcoproporphyrin III (isomer mixture)
Mono, di, tri and tetraglutamylcoporphyrin III
Mono and diaspartylhematoporphyrin IX
Mono and diglutamylhematoporphyin IX

Bacteriochlorin Derivatives

Mono and diaspartylbacteriochlorin c_4

Mono and diglutamylbacteriochlorin c_4

Mono and diaspartylbacterioisochlorin c_4

Mono and diglutamylbacterioisochlorin c_4

Mono, di and triaspartylbacteriochlorin e_6

Mono, di and triglutamylbacteriochlorin e_6

Monoaspartylpyrobacteriopheophorbide a

Monoglutamylpyrobacteriopheophorbide a

Monoaspartylbacteriopheophorbide a

Monoglutamylbacteriopheophorbide a

The aforesaid compounds form salts with either acids or bases. The acid salts are particularly useful for purification and/or separation of the final amide products as are the salts formed with bases. The base salts, however, are particularly preferred for diagnostic and therapeutic use as hereindescribed.

The acid salts are formed with a variety of acids such as the mineral acids, hydrochloric, hydrobromic, nitric and sulfuric acids, organic acids such as toluenesulfonic and benezenesulfonic acids.

The base salts include, for example, sodium, potassium, calcium, magnesium, ammonium, triethylammonium, trimethylammonium, morpholine and piperidine salts and similar such salts.

The acid and base salts are formed by the simple expediency of dissolving the selected amino acid tetrapyrrole amide in an aqueous solution of the acid or base and evaporation of the solution to dryness. The use of a water-miscible solvent for the amide can assist in dissolving the amide.

The final amide products can also be converted to metal complexes for example by reaction with metal salts. The magnesium complexes may be useful for the same purpose as the adduct product. Other metal complexes, as well as the magnesium complex, including, for example, iron and zinc, are useful to preclude contamination during processing of the adduct product by metals such as nickel, cobalt and copper, which are difficult to remove. Zinc and magnesium are readily removed from the final adduct product after processing is completed.

Since many of the aminodicarboxylic acids exist in both the D- and L-forms, and also are employed in mixtures of these forms as well as the D,L-form, the selection of the starting amino acid will, of course, result in products in which the respective isomer or mixture of isomers exist. The present invention contemplates the use of all such isomers, but the L-form is particularly preferred.

The aforesaid compounds are prepared by the usual peptide synthetic routes which generally include any amide-forming reaction between the selected amino acid and the specific tetrapyrrole. Thus, any amide-forming derivative of the tetrapyrrole carboxylic acid can be employed in producing the present new peptides, e.g., lower alkyl esters, anhydrides and mixed anhydrides.

The preferred preparative methods use mixed anhydrides of the carboxylic acid or carbodiimides. The reactants are merely contacted in a suitable solvent therefor and allowed to react. Temperatures up to the reflux temperature can be used, with the higher temperatures merely reducing the reaction time. Excessively high temperatures are usually not preferred so as to avoid unwanted secondary reactions however.

The procedures for forming the instant peptides are well known in this art and are provided in detail in the accompanying examples.

When the selected tetrapyrrole contains more than one carboxyl group, then mixtures of products can be formed including isomeric monopeptide products and di- and even tri- or higher peptide products,

depending on the number of carboxyl groups and depending on the selected stoichiometry. Thus, when equimolar mixtures of amino acid and tetrapyrrole are reacted, not only monopeptides but also dipeptides are obtained, although the monopeptide would predominate. With higher molar ratios, the nature of the products will similarly vary. It is generally possible to separate the monopeptides and higher peptides using known chromatographic techniques. However, such separations are not necessary since the mixed peptides are usually comparable to the separated products in their ultimate use. Thus, mixtures of the mono-, di- and tripeptides of the same tetrapyrrole can be used.

Usually, unreacted tetrapyrrole is separated from the peptide products of the invention during purification as, for example, by chromatographic techniques.

Photodiagnosis and Phototherapy

The compositions of the present invention are useful for the photodiagnosis and phototherapy of tumor, cancer and malignant tissue (hereinafter referred to as "tumor").

When a man or animal having tumor is treated with doses of a compound of the present invention and when appropriate light rays or electromagnetic waves are applied, the compound emits light, i.e., fluorescence. Thereby the existence, position and size of tumor can be detected, i.e., photodiagnosis.

When the tumor is irradiated with light of proper wavelength and intensity, the compound is activated to exert a cell killing effect against the tumor. This is called "phototherapy".

Compounds intended for photodiagnosis and phototherapy ideally should have the following properties:

(a) non-toxic at normal therapeutic dosage unless and until activated by light;

(b) should be selectively photoactive;

(c) when light rays or electromagnetic waves are applied, they should emit characteristic and detectable fluorescence;

(d) when irradiated with light rays or electromagnetic waves are applied, they are activated to an extent to exert a cell killing effect against tumor; and

(e) easily metabolized or excreted after treatment.

In accordance with testing up to the present, the compounds of the present new therapeutic compositions have the foregoing properties and are also characterized by reasonable solubility in water at physiological pH.

The aforesaid compounds possess greater fluorescence in tumors than do the corresponding basic tetrapyrroles, and even peptides formed with amino monocarboxylic acids, e.g., alanine and epsilon aminocaproic acid. Their use provides the best contrast in tumors compared to normal tissue around the tumor. The instant compounds absorb activating energy for phototherapy in the convenient range of 600 to 800 nanometers, with the preferred compounds absorbing in the 620-760 nanometer range, i.e., light of longer wavelengths which more readily permits penetration of energy into the tumor for phototherapeutic purpose.

In present experience, the present compounds more uniformly distribute throughout the tumor than the basic tetrapyrrole permitting the use of considerably

lower dosage (to about 1/10th of the required normal dose of the basic tetrapyrrole) which lessens, if not eliminates, photosensitization in the host. They also possess a more consistent fluorescence whereas some of the corresponding tetrapyrroles show inconsistent fluorescence or the fluorescence varies from day to day in the host.

A particularly advantageous property of the present compounds resides in the ease with which they are excreted by the host. Generally, within 48 to 72 hours of intravenous or intraperitonal administration, there are little or no detectable amounts in normal muscle tissue. The present compounds which are excreted with their chromosphore intact are recovered from the feces of the host within 48-72 hours of injection. Under equivalent circumstances, substantial amounts of the corresponding tetrapyrroles remain, as compared with only minor amounts of peptides formed with the amino monocarboxylic acids remain in the host, e.g., up to about 20%. This property is extremely important in that it contributes to minimization of photosensitization of the host.

The instant composition can be used for diagnosis and therapeutic treatment of a broad range of tumors. Examples of tumors are gastric cancer, enteric cancer, lung cancer, breast cancer, uterine cancer, esophageal cancer, ovarian cancer, pancreatic cancer, pharyngeal cancer, sarcomas, hepatic cancer, cancer of the urinary bladder, cancer of the upper jaw, cancer of the bile duct, cancer of the tongue, cerebral tumor, skin cancer, malignant goiter, prostatic cancer, cancer of the parotid gland, Hodgkins's disease, multiple myeloma, renal cancer, leukemia, and malignant lymphocytoma.

For diagnosis, the sole requirement is that the tumor be capable of selectively fluorescing when exposed to proper light. For treatment, the tumor must be penetrable by the activation energy. For diagnosis, light of shorter wavelength is used whereas for therapeutic purposes light of longer wavelength is used to permit ready penetration of the tumor tissue. Thus, for diagnosis, light of from 360-760 nanometers can be used, and for treatment, from 620 to 760, depending on the individual characteristics of the tetrapyrrole. The absorption characteristics of the present new compounds are substantially the same as the tetrapyrrole from which derived.

It is necessary that the light rays be so intense as to cause the compounds to emit fluorescence for diagnosis and to exert a cell killing effect for therapy.

The source of irradiation for photodiagnosis and phototherapy is not restricted, however, but the laser beam is preferable because intensive light rays in a desired wavelength range can be selectively applied. For example, in photodiagnosis, the compound of the invention is administered to a human or animal body, and after a certain period of time, light rays are applied to the part to be examined. When an endoscope can be used for the affected part, such as lungs, gullet, stomach, womb, urinary bladder or rectum, it is irradiated using the endoscope, and the tumor portion selectively emits fluorescence. This portion is observed visually, or observed through an adapted fiber scope by eye or on a CRT screen.

In phototherapy, after administration of the dosage, the irradiation is carried out by laser beams from the tip of quartz fibers. Besides the irradiation of the surface of tumor, the internal part of the tumor can be irradiated by inserting the tip of quartz fibers into the tumor. The irradiation can be visually observed or imaged on a CRT screen.

For photodiagnosis, light of wavelengths between 360 and 760 nm. is suitable for activating the present tetrapyrrole compounds. Of course, each compound has a specific optimal wavelength of activation. A long wavelength ultraviolet lamp is particularly suitable for photodiagnosis. Similar methods for viewing of the treated tumor can be used as already described for phototherapy.

The dosages of compounds having the present new composition will vary depending on the desired effect, whether for diagnosis or for treatment. For diagnosis, doses of as little as 1 mg/kg will be effective, and up to about 20 mg/kg can be used. For treatment, the dose will usually approximate about 0.5 mg/kg. Of course, the dosage for either diagnosis or treatment can be varied widely in view of aforesaid advantageous properties of the present compounds, e.g., the ease of elimination from the host, for one.

The present compounds are apparently non-toxic at the dosage levels employed for diagnosis or treatment. No mortality of test animals due the present compounds has been noted in studies employing dosage levels up to 20 mg/kg.

For both diagnosis and treatment, the present compounds can be administered by the oral, intravenous, or intramuscular routes. They can be formulated as lyophilized sterile, pyrogen-free compounds, preferably in the form of basic salts, e.g., sodium salt. The preferred dosage forms are provided as injectable solutions (isotonic).

The irradiation source used in treatment of tumors containing compounds of this invention is a filtered, high-intensity, continuous source or pumped dye, or other laser and light delivery system, which is capable of performing within the following limits: power intensity 20-500 mw/cm^2 at wavelengths between 620 and 760 nm. and a total output of at least 500 mw or greater. Several currently commercially available lasers meet these criteria.

The tetrapyrroles can be prepared by various synthetic methods which are found in the literature, e.g.,

Pheophorbides

Willstatter, R., Stoll, A.; Investications on Chlorophyll, (Transl. Schertz, FM.M., Merz, A.R.) p. 249. Science Printing Press, Lancaster, Pennsylvania, 1928.

Pennington, F.C., Strain, H.H., Svec, W.A., Katz, J.J.; J. Amer. Chem. Soc., 86, 1418 (1964).

Chlorin e_6

Willstatter, R., Stoll, A.; Investigations on Chlorophyll, (Trans., Schertz, F.M., Merz, A.R.,) p. 176. Science Printing Press, Lancaster, Pennsylvani, 1928.

Willstatter, R., Isler, M.; Ann. Chem., 390, 269 (1912).

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Conant, J.B., Mayer, W.W.; J. Amer. Chem. Soc., 52, 3013 (1930).

Chlorin e_4

Fisher, H., Heckmaier, J., Plotz, E.; Justus Leibigs Ann. Chem., 500 215 (1933).

Chlorin e_6, c_4, isochlorin c_4, mesochlorin e_6, bacteriopheophorbide, bacteriochlorin e_6

Fischer and Orth, "Des Chemie des Pyrrole" Akademische Verlazsgesellschaft, Leipzig, 1940, Vol. II, Part 2.

General Reference for Porphyrins

"Porphyrins and Metalloporphyrins" ed. Kevin M. Smith, Elsevier 1975 N.Y.

The compounds of the present invention can be administered to the host in a variety of forms adapted to the chosen route of administration, i.e., orally, intravenously, intramuscularly or subcutaneous routes.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 50 and 300 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the

active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. Solutions of the active compound as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporanous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of micro-

organisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The present new compounds may also be applied directly to tumors, whether internal or external, in the host in topical compositions. Exemplary compositions include solutions of the new compounds in solvents, particularly aqueous solvents, most preferably water. Alternatively, for topical application particularly to skin tumors, the present new compounds may be dispersed in the usual cream or salve formulations commonly used for this purpose or may be provided in the form of spray solutions or suspensions which may include a propellant usually employed in aerosol preparations.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatable with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of tumors in living subjects.

EXAMPLE 1

Di (D,L) aspartyl transmesochlorin IX (Carbodiimide Method)

140 mg of transmesochlorin and 200 mg of (D,L) aspartic acid dimethyl ester hydrochloride were dissolved in 30 ml of dimethyl formamide. 300 mg of N,N'-dicyclohexyl-carbodiimide was added. The reaction was allowed to stand for one hour, then another 300 mg of carbodiimide was added. This procedure was repeated twice and then the reaction mixture was allowed to stand overnight. The reaction may be monitored by thin layer chromatography on silica, using solvent benzene/methanol/88% formic acid 8.5/1.5/0.13 V/V/V.

The disubstituted chlorin has the highest R_f value, the unsubstituted chlorin has the lowest, with the monosubstituted isomers in between and unresolved.

After standing overnight, the reaction mixture appeared to contain at least 50% of the disubstituted chlorin. The solvent was removed under vacuum and the remaining solid dissolved in 50 ml of 3N HCl.

The solution was allowed to stand at room temperature for 48 hours to hydrolyze the ester groups, then the chlorin mixture was precipitated at pH 2.5-3 and collected and washed with water at the centrifuge.

The chlorin mixture was purified by dissolving in 0.05 M NH_4OH and applying to a reverse phase (C-18 silica) column 2.5 cm X 30 cm. The elution procedure is a linear gradient from 40 to 70% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The leading green band (di D, L aspartyl trans-mesochlorin IX) was collected and flash evaporated to remove the methyl alcohol, the solution then precipitated at pH 2.5-3 and collected and washed 3 times at the centrifuge with dilute acetic acid. The product was dried under vacuum. The yield was 67 mg of di (D,L) aspartyl transmesochlorin IX.

EXAMPLE 2

Di and Mono (L) glutamyl transmesochlorin IX (mixed anhydride method)

50 mg (0.000087 moles) of transmesochlorin IX was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 195 µl (0.00179 moles) of ethyl-chloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2 M KOH containing 250 mg (0.00169 moles) of (L) glutamic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components. The order of elution was di (L) glutamyl transmesochlorin IX, mono (L) glutamyl transmesochlorin IX, and unsubstituted transmesochlorin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 3

Di and mono (D,L) aspartyl photoprotoporphyin IX (mixed anhydride method)

313.4 mg of photoprotoporphyrin IX (isomer mixture) was dissolved in 100 mls of tetrahydrofuran (THF). 210 µl of triethylamine was added with stirring. After 10 minutes, 210 ul of ethyl chloroformate was added. After stirring for 10 minutes, 50 mls of 0.2 m KOH, containing 450 mgs of (D,L) aspartic acid, were added to the THF solution. This mixture was stirred for one hour at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the pH of the mixture was adjusted to 7.5-8.0 and the solution was placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40/80% MeOH in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components.

The methanol was flashed off and the material was precipitated at pH 3.0-3.5. The ppt was washed 3 times with dilute acetic acid in H_2O. The product was dried under vacuum. The yield of mono(D,L) aspartyl photoprotoporphyrin IX was 54 mg. The yield of di (D,L) aspartyl photoprotoporphyrin IX was 227.8 mg.

EXAMPLE 4

Di and Mono (L) aspartyl protoporphyrin IX
(mixed anhydride method)

100 mg of protoporphyrin IX was dissolved in 100 ml of P-dioxane. 210 µl of triethylamine was added. After stirring 10 minutes, 50 ul of 0.2 M KOH containing 500 mg of (L) aspartic acid was added to the dioxane solution. This mixture was stirred for one hour at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the pH of the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-70% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in H_2O. The product was then dried under vacuum. The yield of mon (L) aspartyl protoporphyrin IX was 12.3 mg and di (L) aspartyl protoporphyrin IX was 54 mg.

EXAMPLE 5

Di and mono (L) aspartyl mesoporphyrin IX

(mixed anhydride method)

200 mg of mesoporphyrin IX was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl of triethylamine was added to the THF solution. After 10 minutes of stirring 210 µl ethyl chloroformate was added and stirred 10 minutes. 50 ml of 0.2 M KOH containing 500 mg of (L) aspartic acid was added to the THF solution and allowed to stir one hour at room temperature.

The organic solvent was flashed off and the reaction mixture was checked for product by silica TLC using benzene/methanol/88% formic acid (8.5/1.5/0.13) to develop the chromatogram.

After checking for product, the pH of the mixture was adjusted to 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components.

The methanol was flashed off and the material was precipitated at pH 3.0-3.5. The ppt was washed 3 times with dilute acetic acid in H_2O. The product was dried under vacuum with a yield of 41.5 mg mono (L) aspartyl mesoporphyrin and 175.1 mg di (L) aspartyl mesoporphyrin.

EXAMPLE 6

Di and Mono (L) aspartyl deuteroporphyrin IX (mixed anhydride method)

100 mg deuteroporphyrin IX was dissolved in 50 ml of p-dioxane. 210 µl of triethylamine was added with stirring. After 10 minutes, 210 µl of isobutyl chloroformate was added. After stirring 10 minutes, 50 ml of 0.2 M KOH containing 500 mg of L aspartic acid was added to the dioxane solution. This mixture was stirred for one hour at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the pH of the mixture was adjusted to 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-70% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components.

The MeOH was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in H_2O. The product was then dried under vacuum. The yield of mono (L) aspartyl deuteroporphyrin IX was 10 mg.

EXAMPLE 7

(L) Aspartyl pyropheophorbide a (mixed anhydride method)

80 mg of pyropheophorbide a was dissolved in 100 ml of tetrahydrofuran (THF) 210 µl of triethylamine was to the THF solution. After 10 minutes of stirring, 210 µl of ethylchloroformate was added and stirred 10 minutes. 50 ml of 0.2 N KOH containing 500 mg of (L) aspartic acid was added to the THF solution and allowed to stir one hour at room temperature.

The organic solvent was flashed off and the reaction mixture was checked for product by silica TLC using benzene (methanol) 88% formic acid (8.5/1.5/0.13) to develop the chromatogram.

After checking for product, the pH of the mixture was adjusted to 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01 M KOH buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components.

The methanol was flashed off and the material was precipitated at pH 3.0-3.5. The ppt was washed 3 times with dilute acetic acid in H_2O. The product was dried under vacuum to produce a yield of 62 mg (L) aspartyl pyropheophorbide a.

EXAMPLE 8

Tetra, tri, and di (D,L) aspartyl coproporphyrin III (mixed anydride method)

150 mg of coproporphyrin III was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl of triethylamine was added and stirring was continued at 20°C for ten minutes. 210 µl of ethylchloroformate was next added and stirred for ten minutes.

50 ml of 0.2 M KOH containing 250 mg of (D,L) aspartic acid was added to the THF solution. This mixture was then stirred for one hour.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC using the following solvent system: (benzene/methanol/88% formic acid (8.5/4.0/0.2).

The pH of this mixture was then adjusted to 7.5-8.0 and chromatographed on a reverse phase (C-18 silica) 2.5x30 cm column. The reaction mixture was resolved using 5-50% methanol in 0.01 in KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components. The methanol was flashed off and the material was precipitated at pH 3.0-3.5. The ppt was washed 3 times with dilute acetic acid in water. The products were dried under vacuum and the yields were as follows: Tetra (D,L) aspartyl coproporphyrin III 94 mg, Tri (D,L) aspartyl coproporphyrin III 77.2 mg, Di (D,L) aspartyl coproporphyrin III, 28.4 mg.

EXAMPLE 9

Di and mono (DL) aspartyl deuteroporphyrin IX (mixed anhydride method)

175 mg (0.00195 moles) of deuteroporphyrin IX was dissolved in 200 ml of tetrahydrofuran (THF). 210 ul (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 210 ul (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2 M KOH containing 200 mg (0.003 moles) of (DL) aspartic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/01.3) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-65% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components. The order of elution was di (DL) aspartyl deuteroporphyrin IX, mono (DL) aspartyl deuteroporphyrin IX, and unsubstituted deuteroporphyrin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 10

Di and mono (DL) aspartyl hematoporphyrin IX (mixed anhydride method)

400 mg (0.0059 moles) of hematoporphyrin IX was dissolved in 50 ml of tetrahydrofuran (THF). 360 µl (0.0034 moles) of triethylamine was added with stirring. After 10 minutes, 340 µl (0.0031 moles) of ethyl-chloroformate was added. After stirring 10 minutes, 10 ml (0.01 moles) of 1 M KOH containing 600 mg (0.0045 moles) of (DL) aspartic acid was added to the THF solution. This mixture was stirred 90 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 20-70% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components. The order of elution was di (DL) aspartyl hematoporphyrin IX, mono(DL) aspartyl hematoporphyrin IX, and unsubstituted hematoporphyrin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 11

Di and mono (D,L) aspartyl protoporphyrin IX (mixed anhydride method)

300 mg (0.00053 moles) of protoporphyrin XI was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 210 µl (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2M KOH containing 450 mg (0.0033 moles) of (D,L) aspartic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5x30 cm. The reaction mixture was resolved using a linear gradient of 40-65% methanol in 0.01M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components. The order of elution was di (D,L) aspartyl protoporphyrin IX, mono (D,L) aspartyl protoporphyrin IX, and unsubstituted protoporphyrin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 12

Mono (DL) aspartyl pyropheophorbide a (mixed anhydride method)

100 mg (0.000187 moles) of pyropheophorbide a was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 miniutes, 210 µl (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2 M KOH containing 200 mg (0.0015 moles) of (DL) aspartic acid was added to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components. The order of elution was mono (DL) aspartyl pyropheophorbide a, and then unsubstituted pyropheophorbide.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 13

Di and mono L-alpha-aminoadipyl transmesochlorin IX

(mixed anhydride method)

500 mg (0.000087 moles) of transmesochlorin IX was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 210 µl (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2 M KOH containing 250 mg (0.00155 moles) of L-alpha-aminoadipic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components. The order of elution was di L-alpha-aminoadipyl transmesochlorin IX, and unsubstituted transmesochlorin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 14

Di and mono (D) aspartyl mesoporphyrin IX (mixed anhydride method)

200 mg (0.00035 moles of mesoporphyrin IX was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 210 µl (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2M KOH containing 500 mg (0.0038 moles) of (D) aspartic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5x30 cm. The reaction mixture was resolved using a linear gradient of 40-48% methanol in 0.01M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components. The order of elution was di (D) aspartyl mesoporphyrin IX, mono (D) aspartyl mesoporphyrin IX, and unsubstituted mesoporphyrin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 15

Di and mono (L) glutamyl mesoporphyrin IX (mixed anhydride method

400 mg (0.007 moles) of mesoporphyrin IX was dissolved in 50 ml of tetrahydrofuran (THF). 360 µl (0.0035 moles) of triethylamine was added with stirring. After 10 minutes, 340 µl (0.0031 moles) ethylchloroformate was added. After stirring 10 minutes, 10 ml (0.01 moles) of 1 M KOH containing 543 mg (0.00369 moles) of (L) glutamic acid was added to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture was resolved using a linear gradient of 25-60% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via fraction collector and the tube contents were pooled according to individual components. The order of elution was di (L) glutamyl mesoporphyrin IX, mono (L) glutamyl mesoporphyrin IX, and unsubstituted mesoporphyrin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 16

Di and mono (D) aspartyl transmesochlorin IX (mixed anhydride method in 1,4 dioxane)

50 mg (0.000087 moles) of transmesochlorin IX was dissolved in 50 ml of 1,4 dioxane. 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 210 µl (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2M KOH containing 500 mg (0.0038 moles) of (D) aspartic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5x30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components. The order of elution was di (D) aspartyl transmesochlorin IX, mono (D) aspartyl transmesochlorin IX, and unsubstituted transmesochlorin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 17

Di and mono (L) aspartyl transmesochlorin IX (mixed anhydride method in tetrahydrofuran)

135 mg (0.00023 moles) of transmesochlorin IX was dissolved in 100 ml of tetrahydrofuran (THF). 210 µl (0.002 moles) of triethylamine was added with stirring. After 10 minutes, 210 µl (0.0019 moles) of ethylchloroformate was added. After stirring 10 minutes, 50 ml (0.015 moles) of 0.3M KOH containing 750 mg (0.0056 moles) of (L) aspartic acid was added dropwise with stirring to the THF solution. This mixture was stirred 60 minutes at room temperature.

The organic solvent was flashed off and the reaction mixture was checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) was used to develop the chromatogram.

After checking for product, the solution was adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5x30 cm. The reaction mixture was resolved using a linear gradient of 40-80% methanol in 0.01M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent was collected via a fraction collector and the tube contents were pooled according to individual components. The order of elution was di (L) aspartyl transmesochlorin IX, mono (L) aspartyl transmesochlorin IX, and unsubstituted transmesochlorin IX.

The methanol was flashed off and the material was precipitated at pH 2.5-3.0. The ppt was washed 3 times with dilute acetic acid in water. The product was dried under vacuum.

EXAMPLE 18

(D,L)Aspartylpheophorbide a (carbodiimide method)

55 mg pheophorbide a was dissolved in 10 ml dimethylformamide. 50 mg (D,L) aspartic acid dimethyl ester dihydrochloride was added, then 100 mg of N,N'-dicyclohexyl-carbodiimide was added. The reaction was allowed to stand in the dark at room temperature for 1 hour, then 50 mg more carbodiimide was added. After standing for 1 additional hour, 50 mg more carbodiimide was added and the reaction allowed to stand in the dark for 12 hours at room temperature.

The solvent was removed under vacuum and the product dissolved in 50 ml 1% KOH in methanol with 0.5 ml H_2O and allowed to stand in the dark at room temperature. The course of the hydrolysis is followed by thin layer chromatography (C-18 plates with solvent 75/25 MeOH/.01M pH 6.85 KPO_4 buffer).

When hydrolysis of the ester groups is essentially complete, the reaction is terminated by addition of a few drops of glacial acetic acid. The methanol is removed under vacuum and the product is dissolved in 20 ml 0.1 M NH_4OH. This solution is placed on a reverse phase (C-18 silica) column (1.5 cm x 30 cm). The elution procedure was a linear gradient from 50 to 80% methanol in 0.01 M KPO_4 buffer pH 6.85 (500 ml total volume).

The leading green-gray band contained the (D,L) aspartylpheophorbide a which was collected, flash evaporated to remove methyl alcohol, and precipitated at pH 3. The precipitate was collected and washed 3 times at the centrifuge with dilute acetic acid. The yield of dry product was 27 mg.

EXAMPLE 19

L-Monoaspartyl chlorin e_6 (carbodiimide method)

150 mg of chlorin e_6 and 250 mg of L aspartic acid di-t.butyl ester hydrochloride were dissolved in 20 ml of dimethyl formamide. There was made a total of 3-100 mg additions of N,N'-dicyclohexyl-carbodiimide at one hour intervals. After 4 hours, the reaction mixture was diluted with 300 ml ether, washed twice with 200 ml H_2O then extracted with 40 ml 1 M KOH. The KOH solution was allowed to hydrolyze overnight, then heated to 70°C. for 10 minutes.

The pH of the solution was adjusted to 7, then any residual ether was removed by flash evaporation. The solution was then applied to a reverse phase (C-18 silica) column (1.5 cm x 30 cm). The product was purified by a stepwise elution of methanol/.01 M pH 6.85 KPO_4 buffer. Eluted with 5% methanol until unwanted polar pigments were removed. Monoaspartyl chlorin e_6 was eluted off with 6-8% methanol, and unreacted chlorin e_6 was removed with 25% methanol.

The product was precipitated at pH 3 after flash evaporating briefly to remove methanol, then washed at the centrifuge 3 times with dilute acetic acid.

The product was dried under vacuum. Yield of L-monoaspartylchlorin e_6 was 50 mg.

EXAMPLE 20

L Glutamyl chlorin e_4 (carbodiimide method)

110 mg chlorin e_4 and 220 mg L-glutamic acid dimethyl ester hydrochloride were dissolved in 15 ml of dimethyl formamide. 85 mg of N,N'-dicyclohexyl carbodiimide was then added, and the solution stirred for 1 hour at room temperature. 42 mg more carbodiimide was then added, then 50 mg of carbodiimide was added at 1 hour intervals for two more additions. The reaction mixture was then allowed to stand for 12 hours, one more 50 mg carbodiimide addition was made, and the reaction allowed to stand for 3 hours. Progress of the reaction was followed by reverse phase thin layer chromatography 80% methanol, 20% KPO_4 buffer (.01M pH 6.85). A further addition of 50 mg of carbodiimide, with standing, showed no further product formation.

200 ml of ether was added to the reaction mixture, and the ether solution was washed 4 times with water, approximately 100 ml per wash. The ether was then removed by flash evaporation, and the product was dissolved in approximately 25 ml of 3N Hcl. After 48 hours at room temperature, the solution was adjusted to pH3 with NH_4OH, and the precipitate was collected and washed at the centrifuge. The product was dissolved in 20% methanol/ water with a little NH_4OH, and applied to a reverse phase (C-18 silica) column (1.5x30 cm). Elution was continued with 20% MeOH, KPO_4 buffer (0.01M pH 6.85). This removed the product (L-Glutamyl chlorin e_4). The methanol concentration was increased to remove the unreacted chlorin e_4.

The solution was flash evaporated until the methanol was substantially removed, then the products were precipitated at pH3 by addition of Hcl, collected and washed at the centrifuge with dilute acetic acid and dried under vacuum. Yield of mono-L-glutamyl chlorin e_4 21 mg. Yield of recovered chlorin e_4 59 mg.

EXAMPLE 21

L-Monoglutamyl chlorin e_6 (carbodiimide method)

130 mg of chlorin e_6 and 260 mg L glutamic acid dimethyl ester hydrochloride was dissolved in 18 ml of dimethylformamide. 100 mg of N,N'-dicyclohexyl-carbodiimide was added and the reaction mixture stirred for 1 hour. 50 mg more carbodiimide was then added. After 1 hour, the reaction mixture appeared to contain 75-80% of the monosubstituted product by reverse phase TLC (C-18 plates with 70% MeOH, 30% .01 M KPO_4 pH 6.85). 200 ml Diethyl ether was added, washed twice with 100 ml H_2O, then extracted with 30 ml 1 M KOH.

The product was allowed to hydrolyze in the dark in the KOH solution for 12 hours, then was heated to 70°C for 10 minutes, to complete the hydrolysis of the ester groups. The product was then separated by reverse phase column chromatography (C-18 reverse phase silica 1.5 cm x 30 cm), using stepwise gradient elution with methanol in buffer .01 M KPO_4 pH 6.85. 5% Methanol removed polar impurities. The monoglutamyl chlorin e_6 was eluted with 6-8% methanol. Chlorin e_6 was eluted off the column with 25% methanol. The methanol was removed by flash evaporation and the L-monoaspartyl chlorin e_6 was precipitated at pH 3, collected and washed 3 times at the centrifuge with dilute acetic acid, and dried under vacuum. Yield 40 mg.

EXAMPLE 22

Mono and Di (L) Aspartyl Chlorin e_6 Carbodiimide Method)

400 mg of chlorin e_6 and 1 g of L-aspartic acid dibenzyl ester p-tosylate were dissolved in 75 ml of dimethyl formamide. Temperature of the solution was maintained at 65-70°C. with stirring and 100 mg of N,N'-dicyclohexyl carbodiimide was added. (A total of 3 additions were made at 2 hour intervals). The solution was allowed to stir at this tmperature for a total of 20 hrs., then checked by TLC (reverse phase) (C-18 silica) plate, 70% methanol, 30% .01 M pH 6.85 KPO_4 buffer. The TLC showed greater than 50% monosubstitution with some di-substitution.

150 ml of ether was added, and agitated with 100 ml of water and several drops of glacial acetic acid. The ether phase was separated and the aqueous phase extracted several more times with 100 ml of ether. The ether extracts were combined and washed with water (100 ml) four times to remove dimethyl formamide.

The aspartyl chlorin e_6 esters were then extracted into 100 ml of 1 M KOH (4 extractions of 25 ml each). The KOH solution was allowed to stand at ambient temperature for 24 hours to hydrolyze. The components were separated by neutralizing the solution of pH 7 and applying to a reverse phase (C-18 silica) column (1.5 cm x 30 cm). The elution was performed using a 1 liter gradient of 30 % methanol to 80% methanol with 0.1 M pH 6.85 KPO_4 buffer. Fractions were collected and characterized by TLC. The order of elution was di (L) diaspartyl chlorin e_6, L-monoaspartyl chlorin e_6 and

chlorin c_6. Methanol was removed was flash evaporation and the individual components precipitated at pH 3, using HCl.

The products were collected by centrifugation, washed several times with very dilute acetic acid and drived under vacuum. Yield was 23.8 mg.

Physical characteristics of representative compounds (relative polarity) is measured by a standard chromatographic system.

TLC Plate Baker Si-C18 20 um particle size 200 mm coating thickness
Solven System 75% methanol 25% 0.01 M KNO_3 buffer pH 6.85

Compound	Derivative	R_f	Compound	Derivative	R_f
			Trans-mesochlorin IX		
Mesoporphyrin IX	-	.32	"	mono(L)glutamyl	.54
"	mono(D,L)aspartyl	.53	"	di(L)glutamyl	.72
"	di(D,L)aspartyl	.67	deuteroporphyrin IX	-	.55
"	di(D)aspartyl	.66	"	mono(D,L)aspartyl	.75
"	mono(L)aspartyl	.55	"	di(D,L)aspartyl	.65
"	di(L)aspartyl	.66	"	mono(L)aspartyl	.75
"	mono(D,L)glutamyl	.55	"	di(L)aspartyl	.84
"	di(D,L)glutamyl	.72	protoporphyrin IX	-	.33
			"	mono(L)aspartyl	.56
Trans-mesochlorin IX	-	.28	"	di(L)aspartyl	.73
"	mono(D)aspartyl	.52	phetoprotoporphyrin IX (isomer mixture)	-	.58
"	di(D)aspartyl	.64	"	mono(D,L)aspartyl	.78
"	mono(L)aspartyl	.53	"	di(D,L)aspartyl	.85
"	di(L)aspartyl	.64	"	mono(L)aspartyl	.76
Hematoporphyrin IX	-	.78	"	di(L)aspartyl	.85
"	mono(D,L)aspartyl	.88	pyrophcophorbide a	-	.07
"	di(D,L)aspartyl	.89	"	(D,L)aspartyl	.22
Chlorin e_6	-	.66	"	(L)aspartyl	.23
			Mesoporphyrin IX	-	
"	mono(L)aspartyl	.77	"	di(L)glutamyl	.69
"	di(L)aspartyl	.84	"	mono(L)glutamyl	.55
"	mono(L)glutamyl	.79	protoporphyrin IX	-	
Chlorin e_4	-	.57	"	di(D,L)aspartyl	.70
"	mono(L)glutamyl	.74	"	mono(D,L)aspartyl	.57
Trans-mesochlorin IX	-		Coproporphyrin III		.91
"	di(D,L)aspartyl	.67	"	mono(D,L)aspartyl	.92
			"	di(D,L)aspartyl	.93
			"	tri(D,L)aspartyl	.95
			"	tetra(D,L) aspartyl	.97

The visible absorption spectrum in pyridine for all of the aminodicarboxylic acid derivatives are identical to the parent porphyrin, chlorin or bacteriochlorin.

Comparative Spectroscopic Absorption Data

Solvent in All Cases is P-dioxane.

Compounds	Absorption Maxima (nm) in Visible Region	mM Extinction Coefficient (EmM) ± 10%	Soret Band nm	mM Extinction Coefficient (EmM) ± 10%
Photoprotoporphyrin IX isomer mixture	668	38	415	180
Pheophorbide a	667	35	408.6	88
Pyropheophorbide a	668	38	411.2	89
L-aspartylpyropheophorbide a	668.5	47	412.6	112
Trans-mesochlorin IX	643	60	388	183
Di (L) aspartylmesochlorin IX	643.3	53	388.6	160
Mono (D) aspartylmesochlorin IX	643.4	57	388.1	165
Mono (L) aspartylmeschllorin IX	643.6	59	388.3	178
Hematoporphyrin derivative (HPD)	626	2.9	399	102
Chlorin e_6	665.6	42	402	124
Mono (L) aspartyl chlorin e_6	663.5	38	401.7	111
Bacteriopheophorbide a	753.5	44.7	359	76

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The preparation of pharmacological dosages for the administration of the active ingredient, that is the amino acid porphyrin adducts, which were prepared in Examples 1-22 hereinabove, is as follows:

EXAMPLE 23

A tablet base was prepared by blending the following ingredient in the proportion by weight indicated:

	Grams
Sucrose, USP	80.3
Tapioca Starch	13.2
Magnesium Stearate	4.4

Into this base, there was blended sufficient amino acid porphyrin adducts to provide tablets each containing 100 mg. of active indgredient.

EXAMPLE 24

A blend was prepared containing the following ingredients:

	Grams
Calcium phosphate	17.6
Dicalcium phosphate	18.8
Magnesium trisilicate, USP	5.2
Lactose, U.S.P.	5.2
Potato Starch	5.2
Magnesium Stearate A	0.8
Magnesium Stearate B	0.32
Porphyrin Amino Acid Adducts	20

This blend was divided and formed into capsules each containing 25 mg of active ingredient.

EXAMPLE 25

To a commercially available raspberry flavored sugar syrup is added to the equivalent of 40 mg of the amino acid porphyrin adduct per milliliter and the mixture is homogenized in a mechanical device for this purpose. This mixture is especially suitable for oral administration containing 200 mg of the active ingredient.

EXAMPLE 26

A sterile solution of the following composition is prepared: 200 mg of the sodium salt of the amino acid porphyrin adduct is dissolved in a 0.9% NaCl solution so that the final concentration is 20 mg/ml.

This solution is suitable for I.V. and I.M. administration.

EXAMPLE 27

The sodium salt of the amino acid porphyrin adduct is dissolved in 0.9% NaCl solution so that the final concentration is 5 mg/ml. This is place in an aerosal dispenser with a hydrocarbon propellant. This preparation is suitable for topical application.

EXAMPLE 28

PREPARATION OF A METAL SALT

The sodium salt of the porphyrin amino acid adduct is prepared by dissolving said adduct in water containing an equimolar amount of sodium hydroxide and freeze drying the resulting mixture.

In this fashion, other metal salts are prepared including potassium, calcium, and lithium salts.

PREPARATION OF AN ACID SALT

The amino acid porphyrin adduct described in the preceding examples are converted to acid salts, e.g., hydrochloride, by dissolving in an aqueous solution containing

an equivalent amount of acid, e.g., hydrochloric acid, and the solution is evaporated to dryness to obtain the solid salt. Alternately, alcoholic solutions of hydrogen chloride gas, dissolved in ethanol can be used in lieu of the aqueous acid solution and the acid salt is obtained by evaporation of the solvent or crystallization from the alcohol, e.g., by addition of a non-solvent.

The following protocols describe the procedure for the utilization of these new compounds of the present invention in the treatment of rat tumors.

EXAMPLE 29

The photodynamic therapy experiments have been carried out using the compound mono- (L)-aspartyl chlorin e_6. Two transplantable tumor lines in Buffalo rats have been used, Morris Hepatoma 7777 and Morris Hepatoma 5123 tc. The tumors were transplanted subcutaneously on the outside of the thigh. During treatment, the tumors ranged in size between 1 and 2.5 cm in diameter.

The general treatment regime is as follows. The rats are injected with a solution of the chlorin prepared as follows: 20 mg of the sodium salt of the chlorin was dissolved in 1 ml of 0.9% NaCl. The chlorin solution was then injected intravenously through the external jugular while the rat was anesthetized with ether. The volume of solution injected was calculated based upon the weight of the animal and the dosage, on a weight to weight basis, for the particular experiment. A specified time interval was then allowed to elapse before light treatment was instigated.

Light treatment of the rats was without anesthesia. The rats were restrained, the hair removed in the treatment area and treated with laser light from a Cooper Aurora argon pumped, tunable dye laser.

The laser was equipped with a fiber optic light delivery system coupled to a microlens system developed by Dr. Daniel Doiron, D.R.D. Consulting, Santa Barbara, California.

The lens disperses the laser beam, providing a circular distribution of light with homogenous light intensity throughout the area of the incident light beam. The wavelength of light was adjusted using a Hartridge reversion spectroscope. The light intensity was determined using a Yellow Springs Instrument, Model 65A, radiometer.

The micro lens was positioned at such a distance from the skin of the animal so as to provide an illumination diameter of 1.5cm, and the light flux was varied by control of the laser output.

Subsequent to illumination, the animal was returned to its cage and, 24 hours later, it was treated intravenously in the external jugular vein with 14 mg of Evans Blue dye, dissolved in 250 μl of 0.9% NaCl. Two hours after injection, the rat was sacrificed and the tumor cross-sectioned. The extent of tumor necrosis was assessed by the lack of dye uptake [1], and the depth of the necrotic cross section of the tumor was recorded in millimeters.

Table II summarizes the effects of these drugs on tumors and includes a range of wavelengths, dosages, intensities, and time intervals for treatment. This has been necessary, in order to attempt to establish the optimal conditions for phototherapy utilizing this new drug. The conditions described result in measurable and significant damage to the tumors.

In all cases except where noted, tissue damage occurred selectively to the tumor tissue as assayed by the Evans Blue method, even though, in nearly all cases, normal skin overlayed the tumor and the treatment area overlapped significant areas of normal muscle tissue.

(1) M.C. Berenbaum, Br. J. Cancer 45: 571(1982)

The photodynamic therapy date is presented in tabular form. Column No. 2 is the total light dose administered in terms of Joules per square centimeter. Column No. 3 is the dose of mono(L)aspartyl chlorin e_6 administered in terms of mg of drug per kilogram of rat body weight. Column No. 4 is the time lapse between administration of drug and treatment with laser light. Column No. 5 is the wavelength of treatment light in nanometers. Column No.6 is the intensity of the treatment light in milliwatts per square centimeter. In Column No. 7, $\bar{x}$ is the mean depth of necrosis in millimeters of the tumor tissue, i.e., the distance from the necrotic top of the tumor next to the skin to the necrotic edge of the tumor most distant from the skin.

S.D. is the standard deviation of $\bar{x}$.

(N) is the number of tumors or legs involved in the experiment.

Column No. 8 is the range of depth of necrosis in millimeters within the group.

TABLE II

tumor	joules/ cm2	drug dose mg/kg	time in hrs. btwn drug & light	wave-lnth nm	intensity mW/cm2	$\bar{x}$	s.d.	(n)	range mm
7777	10	20	24	655	100	2.8	± 1.6	(10)	1-6
	10	20	24	665	200	2.8	± 1.0	(3)	2-4
	10	20	24	650	200	2.9	± 1.1	(5)	1.5-4.5
	10	20	24	660	100	4.6	± 1.9	(7)	2.5-8
	10	20	24	660	200	3.6	± 1.4	(6)	1-6
	10	20	24	665	100	5.9	± 2.4	(7)	2.5-9
	10	20	48	655	200	7.5	± 4.2	(2)	4.5-10.5
	20	20	24	655	100	4.1	± 1.3	(17)	2-6
	20	20	24	660	100	5.4	± 1.9	(8)	2-7.5
	28.4	20	24	655	200	5.4	± 1.6	(29)	2.5-11
	28.4	15	24	655	200	4.0		(2)	
	56.8	15	24	655	200	4.5	± 0.7	(2)	4-5
	56.8	20	24	655	200	4.5	± 0.7	(2)	4-5
	113	15	24	655	200	Damage Non Specific		(3)	
	113	20	24	655	200	4.0	± 1.2	(4)	3-5
	113	5	48	655	200	3.8	± 1.2	(6)	2-5
	169	15	24	655	200	Damage Non Specific		(5)	
	169	20	24	655	200	5.0		(2)	
	169	5	48	655	200	4.8	± 0.8	(6)	3.5-6
5123tc	10	20	24	655	100	4.0		(1)	
	20	20	24	655	100	2.7	± 1.0	(7)	1-4
	20	20	48	655	200	2.0		(2)	
	20	20	24	665	100	4.9	± 1.0	(8)	3.5-4
	20	20	24	655	200	4.3	± 1.1	(8)	2.5-6 2/10*
	28.4	20	24	655	100	4.1	± 1.3	(4)	3-6 1/5*
	28.4	20	24	655	200	3.2	± 0.3	(3)	3-3.5 4/7*
	30	20	24	655	100	4.4	± 1.0	(4)	3.5-5.5
	56.8	20	24	655	200	3.0		(1)	
	56.8	5	48	655	200	No effect		(9)	
	113	5	48	655	200	No effect		(8)	

*No effect

Control Rats: No Tumor

10 20 24 665 100 (10)

24 hr Evaluation: 5 showed some increased dye uptake in the skin at point of treatment.

20 20 24 665 100 (10)

24 hr Evaluation: 6 showed some increased dye uptake in the skin at point of treatment.

10 20 24 665 100 (10)

14 day Evaluation: none showed signs of skin or tumor necrosis and hair had regrown normally.

20 20 24 665 100 (10)

14 Evaluation: one leg of one animal showed some sign of muscle necrosis. Skin appeared normal and hair regrew normally on all animals.

EXAMPLE 30

PDT Experiments with Mice and mono-L-Aspartyl Chlorin e_6

PDT with the mono-L-aspartyl chlorin e_6 tetrasodium salt was evaluated in another animal/tumor model system.

The tumor, SMT-F, was transplanted subcutaneously into the shoulder/rib area (one side only) of DBA/2 Ha ROS D+ Ha mice. The treatment regime was started when the tumors had reached a dimension of approximately 1.5 - 2 cm long by 1 cm wide and 0.7 to 1 cm deep, (approximately 7 to 8 days after transplant). The drug was administered through intraperitoneal injection at a concentration of 4 mg/ml. Specific parameters and results are listed in the following table. The evaluation was done 24 hours after light treatment using the vital stain Evans Blue in a procedure similar to that which was used for evaluating tumor necrosis in the Buffalo rats, the only difference being the intraperitoneal injection of the dye at a dose of 5 mg per mouse. The headings of each column are the same as the rat system.

Joules/cm^2	Drug Dose mg/kg	Time in Hrs btwn drug & light	Wave-length nm	Intensity mW/cm^2	$\bar{X}$ (mm)	s.d.	(n)	$\bar{D}$ (mm)	s.d.
40	40	24	665	100	6.6±	2.0	7	10.3±	3.

No indication of necrosis of normal tissue (muscle or skin) was observed.

Similar results are obtained when the compounds in Examples 1-22 are administered to a similarly pretreated mice.

EXAMPLE 31

PDT Experiments with Rats and mono-L-Glutamyl Chlorin e_6

Buffalo rats with Morris Hepatoma 7777 transplanted subcutaneously on the outside of each hind leg were subjected to photodynamic therapy, using mono-L-glutamyl chlorin e_6 tetrasodium salt as the drug.

The experimental procedure was the same as is employed for testing of the mono-L-aspartyl chlorin e_6. Specific parameters and results are listed in the table below.

No visible damage - as assessed by the Evans Blue method - to the overlying skin or normal muscle tissue surrounding the tumor was observed, although the 1.5 cm diameter area of light treatment overlapped normal tissue in several cases.

Column No. 1 is the total light dose administered in terms of Joules per square centimeter. Column No. 2 is the dose of chlorin administered in terms of mg of drug per kilogram of rat body weight. Column No. 3 is the time lapse between administration of drug and treatment with laser light. Column No. 4 is the wavelength of treatment light in nanometers. Column No. 5 is the intensity of the treatment light in milliwatts, per square centimeter. In Column No. 6, X is the mean depth of necrosis in millimeters of the tumor tissue, i.e., the distance from the necrotic top of the tumor next to the skin to the necrotic edge of the tumor most distant from the skin. s.d. is the standard deviation of X, (n) is the number of tumors or legs involved in the experiment. D is the mean diameter of tumor necrosis with the following s.d. the standard deviation for D.

Joules/cm^2	Drug Dose mg/kg	Time in Hrs btwn drug & light	Wave-length nm	Intensity mW/cm^2	$\bar{X}$ (mm)	s.d.	(n)	$\bar{D}$ (mm)	s..
20	20	24	665	100	3.4	± 1.3	17	9.6	±

Similar results are obtained when Compounds 1-22 of the preceding examples are administered to similarly pretreated rats.

EXAMPLE 32

PDT EXPERIMENTS WITH MICE AND MONO-L-ASPARTYL CHLORIN e_6

The SMT-F tumor in DBA/2 Ha ROS D+ Ha mouse system was used to evaluate the photodynamic effect of mono-L-glutamyl chlorin e_6 tetrasodium salt.

The protocol is the same as the experiment involving mono - L - aspartyl chlorin e_6, and the column headings are the same as those used in this system and the rat system.

Joules/ cm^2	Drug Dose mg/kg	Time in Hrs btwn drug & light	Wave-length nm	Intensity mW/cm^2	$\bar{X}$ (mm)	s.d.	* (n)	$\bar{D}$ (mm)	s.d.
40	40	24	665	100	7.9	± 2.9	8	13.9	±3.5

* A ninth mouse showed no response and was not included in the above statistical analysis. This is because of the possibility that drug was injected into the gut instead of the peritoneum.

EXAMPLE 33

Human cells (HeLa, strain D98/AH2) were incubated in 25 cm^2 plastic culture flasks for 24 h to permit attachment. They were then rinsed, incubated for 10 minute periods in Ham's F-12 medium containing porphyrins, rinsed again in Ham's F-12 medium without porphyrins for 5 minutes, then illuminated for various periods, and cultured at 37°C in complete medium for 24 h. Cell counts were then made using a phase contrast microscope of the fraction of the surviving cells. The broad band incandescent light source used was adjusted to give an incident light intensity of 5×10^5 erg cm^{-2} sec^{-1}. A positioning device permitted illuminating each of five areas of a flask for different times; one area was not illuminated and served as a dark control. This gave a four light dose survival curve from a single flask; the technique is thus suitable for the rapid and economical screening of large numbers of potential photosensitizing agents. The results of this experiment are shown in Table III.

TABLE III

Sensitizer	Percent of Cells Surviving 24 Hours After Illumination Time of Illumination in minutes						
	0	.35	.75	.15	3	5	8
Mesoporphyrin IX mono-L-aspartic acid	100	96	0	0.4	0	0	0
Mesoporphyrin IX di-L-aspartic acid	100	100	100	100	92	0	0
Mesochlorin IX mono-L-glutamic acid	100	46	0	0	0	0	0
Mesochlorin IX di-L-glutamic acid	100	100	100	100	98	92	0
Chlorin e_6 mono-L-aspartic acid	100	99	98	82	1	0	0

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TABLE III CON'T

HeLa CELL STUDIES

Sensitizer	Per Cent of Cells Surviving 24 hrs. after Illumination. Period of Illumination (min.) 0	0.35	0.75	1.5	3.0	5.0
Di aspartyl mesoporphyrin IX	100	100	100	98	5	0
Aspartyl pyropheophorbide a	100	100	0	0	0	0
Aspartyl pyropheophorbide a (same solution as above, kept in refrigerator)	100	100	0	0	0	0

Two tenths ml of $4x10^{-4}$ M solution (or suspension) of the sensitizer were mixed with 1.8 ml of Ham's medium for the experiments - thus the cells were treated with $4x10^{-5}$ M of sensitizer. The cells were incubated for 10 minutes in the presence of sensitizer, then washed for 5 minutes in Ham's without sensitizer and then illuminated in Ham's for the time indicated.

EXAMPLE 34

SCREENING OF PORPHYRIN FLUORESCENCE AS A FUNCTION OF MOLECULAR STRUCTURE

Two transplantable tumor lines in Buffalo rats were used, Morris Hepatoma 7777 and Morris Hepatoma 5123tc. The tumors were transplanted intramuscularly on the rear of the thigh of the rats. After 10-14 days, when the tumors reached the appropriate size, 2 mg (0.5 ml) of an amino acid porphyrin adduct solution were introduced intraperitoneally into the rats. The amino acid porphyrin adduct solution was prepared as follows: 4 mg of the amino acid porphyrin was dissolved in 0.1 M NaOH and adjusted to physiological pH with 1 M HCl.

The rats were killed 24 hours after the injection. The tumor was bisected in situ. The porphyrin fluorescence was determined under a constant intensity UV light source.

Tables IV, V, VI and VII list the porphyrin derivatives tested. The compounds are grouped alphabetically.

Following the name of the porphyrin is a number that indicates the total number of tumors examined. The next column of figures (A) is a number calculated as follows: the porphyrin fluorescence within the tumor was ranked visually by one person under a constant intensity U.V. light source according to the scale 0, +½, 1, 2, 3, 4. This number was then multiplied by the percent of the tumor demonstrating this fluorescence, i.e. (+½) (80%) + (+1) (10%) = 50. More often than not, the A value in the table represent averages obtained in several series of separate experiments conducted at different times.

The "C value" for each tumor is the "A value" for that tumor divided by the average diameter of the tumor, in cm.

A time study of 12-72 hours was also conducted on some of the tumors. The procedure is the same as above, except 1 mg of the amino acid adduct was utilized. The results are also indicated in Table IV.

TABLE IV

SCREENING EXPERIMENTS*

Porphyrin Derivative	Dsg	Time	# of Tumors	Avg Diam	% Fluor	A	B	C
Chlorin e_6 mono-L-glutamyl	2mg	24hr	17	2.41	90	67	12	28
Chlorin e_6 mono-L-aspartyl	2mg	24hr	18	2.51	88	74	12	30
Chlorin e_6 mono-L-aspartyl	2mg	24hr	10	1.5	90.6	46.2	31.9	38.5
Control			4	3.05	29	15	2	5
Control			2	2.6	57.5	32.5	12.5	20.3
Chlorin e_6 mono-L-glutamyl	2mg	24hr	16	2.7	59.9	34.7	12.9	21.1
TIME STUDY								
Chlorin e_6 mono-L-aspartyl	1mg	12hr	6	2.2	81.7	42.1	19.1	20.1
Chlorin e_6 mono-L-aspartyl	1mg	18hr	6	1.3	95	47.7	36.7	43.4
Chlorin e_6 mono-L-aspartyl	1mg	36hr	6	1.8	93.5	61.8	34.3	47.5
Chlorin e_6 mono-L-aspartyl	1mg	48hr	6	1.5	80.8	42.1	28.4	35.1
Chlorin e_6 mono-L-aspartyl	1mg	72hr	6	1.7	89.2	48.3	28.4	37.2

*Tumor - Morris Hepatoma 7777

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TABLE V
TUMOR LINE: MORRIS HEPATOMA 7777
CUMULATIVE TABLE FOR 2mg DOSE
24hr EXAMINATION

PORPHYRIN	DERIVATIVE	TUMORS	A	C
Mesoporphyrin IX	mono (D,L) aspartyl	16	31	17
Mesoporphyrin IX	di (D,L) aspartyl	20	56	27
Mesoporphyrin IX	di (D,L) aspartyl	10	59	33
Mesoporphyrin IX	di (D,L) aspartyl	20	39	19
Mesoporphyrin IX	di (D,L) aspartyl	16	54	40
Mesoporphyrin IX	di (D) aspartyl	19	32	15
Mesoporphyrin IX	di (L) aspartyl	20	53	25
Mesoporphyrin IX	mono (L) glutamyl	20	21	10
Mesoporphyrin IX	di (L) glutamyl	20	39	13
Mesoporphyrin IX	di (L) glutamyl	30	60	30
Protoporphyrin IX	mono (D,L) aspartyl	20	5	3
Protoporphyrin IX	di (D,L) aspartyl	20	33	17
Protoporphyrin IX	di (L) aspartyl	20	36	23
Photoprotoporphyrin IX	Di (D,L) aspartyl	20	7	3
Photoprotoporphyrin IX	mono (D,L) aspartyl	18	18	10

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TABLE V (Con't)
TUMOR LINE: MORRIS HEPATOMA 7777
CUMULATIVE TABLE FOR 2mg DOSE
24hr EXAMINATION

PORPHYRIN	DERIVATIVE	TUMORS	A	C
Coproporphyrin IX	mono (D,L) aspartyl	10	27	13
Coproporphyrin IX	mono (D,L) aspartyl	16	38	17
Coproporphyrin IX	di (D,L) aspartyl	4	21	12
Coproporphyrin IX	di (D,L) aspartyl	19	12	6
Coproporphyrin IX	tri (D,L) aspartyl	20	19	9
Coproporphyrin IX	tetra (D,L) aspartyl	20	4	1
Mesochlorin IX	di (D,L) aspartyl	12	30	16
Mesochlorin IX	di (D,L) aspartyl	20	39	20
Mesochlorin IX	di (L) aspartyl	28	41	24
Pheophorbide a	(L) aspartyl	20	23	14
Pryopheophorbide a	(D,L) aspartyl	11	30	13
Pyropheophorbide a	(D,L) aspartyl	10	41	17
Pyropheophorbide a	(D,L) aspartyl	6	25	10
Pyropheophorbide a	(D,L) aspartyl	16	23	13
Pyropheophorbide a	(L) aspartyl	6	45	13

TABLE V (Con't)
TUMOR LINE: MORRIS HEPATOMA 7777
CUMULATIVE TABLE FOR 2mg DOSE
24hr EXAMINATION

PORPHYRIN	DERIVATIVE	TUMORS	A	C
Chlorin e_6	mono (L) aspartyl	18	74	30
Chlorin e_6	mono (L) glutamyl	18	67	23

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TABLE VI
TUMOR LINE: MORRIS HEPATOMA 7777

PORPHYRIN	DERIVATIVE	# OF TUMORS	AVG DIAM OF TUMORS (cm)	% FLUORS	A	C
Mesoporphyrin IX	di (D,L) aspartyl	20	1.97	61	39	20
Mesoporphyrin IX	di (D,L) aspartyl 168 hrs	4	1.97	72	38	12
Mesoporphyrin IX	di (L) aspartyl	20	2.07	79	53	26
Mesoporphyrin IX	di (L) aspartyl	14	1.14	66	40	35
Mesoporphyrin IX	di (D) aspartyl	20	2.14	48	32	15
Mesochlorin IX	mono (D,L) aspartyl, mono methyl ester	10	1.22	28	20	17
Mesochlorin IX	mono (D,L) aspartyl, mono methyl ester	6	1.73	21	15	9
Mesochlorin IX	di (D,L) aspartyl	20	1.95	65	39	20
Mesochlorin IX	di (L) aspartyl	28	1.70	60	41	24
Mesochlorin IX	di (L) aspartyl	12	1.85	53	30	16
Deuterochlorin IX	di (D.L) aspartyl	8	2.02	59	32	16
Chlorin e_6	mono (L) aspartyl	18	2.51	88	74	30
Chlorin e_6	mono (L) glutamyl	17	2.41	90	67	28
Chlorin e_4	mono (L) glutamyl	16	1.64	60	35	22

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TABLE VI (Con't)
TUMOR LINE: MORRIS HEPATOMA 7777

PORPHYRIN	DERIVATIVE	# OF TUMORS	AVG DIAM OF TUMORS (cm)	% FLUORS	A	C
Methyl pyroporphyrin XXI	(D,L) aspartyl	12	1.97	8	4	2
Pheophorbide a	(D,L) aspartyl	6	2.63	43	25	10
Pheophorbide a	(L) aspartyl	20	1.61	45	23	14
Pyropheophorbide a	(D,L) aspartyl	16	1.79	44	23	13
Pyropheophorbide a	(D,L) aspartyl	4	2.80	69	37	13
Photoprotoporphyrin IX	di (D,L) aspartyl 10mg Hemin 1 hr prior	6	1.76	26	15	8
Mesoporphyrin IX	di (D,L) aspartyl 10mg Hemin 1 hr prior	6	1.58	69	43	27

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TABLE VII
TUMOR LINE: MORRIS HEPATOMA 5123TC

PORPHYRIN	DERIVATIVE	# OF TUMORS	AVG DIAM OF TUMORS (cm)	% FLUORS	A	C
Mesoporphyrin IX	di (L) aspartyl	10	1.98	84	39	20
Mesoporphyrin IX	di (L) aspartyl	20	1.14	43	25	22
Mesoporphyrin IX	di (D) aspartyl	16	1.34	25	16	12
Mesoporphyrin IX	di (D,L) aspartyl	20	1.14	14	9	8
Mesoporphyrin IX	di (D,L) aspartyl	8	1.48	39	23	16
Mesochlorin IX	mono (D,L) aspartyl	8	1.48	65	33	22
Mesochlorin IX	mono (D,L) aspartyl mono-isoamyl ester	6	1.41	95	48	34
Mesochlorin IX	di (D,L) aspartyl	12	1.52	27	7	5
Mesochlorin IX	di (D,L) aspartyl	20	1.18	3	4	3
Mesochlorin IX	di (D,L) aspartyl	8	1.67	18	10	6
Mesochlorin IX	di (D) aspartyl	19	1.61	39	20	12
Mesochlorin IX	di (L) aspartyl	20	1.27	40	25	20
Protoporphyrin IX	di (D,L) aspartyl	8	1.45	35	21	14
Pyropheophorbide a	(L) aspartyl	18	2.07	6	3	2
Pyropheophorbide a	(D,L) aspartyl	8	1.27	39	20	15
Chlorin e_6	mono (L) aspartyl	11	1.10	26	26	24
Chlorin e_6	mono (L) glutamyl	13	1.34	24	12	10

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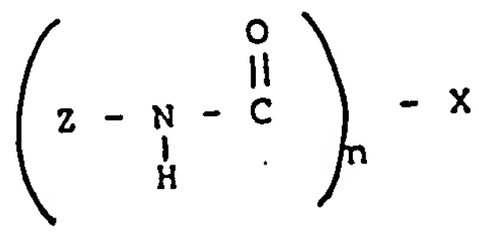
CLAIMS

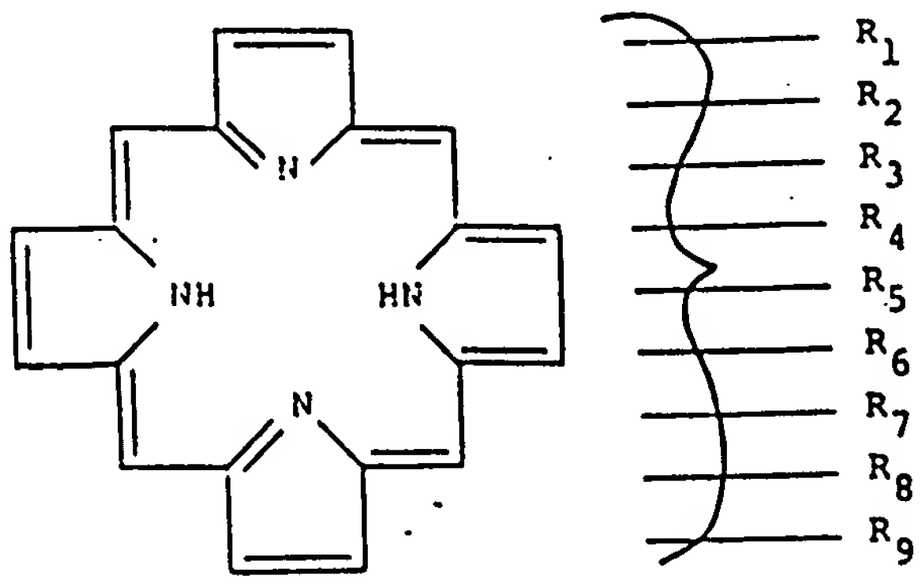
1. A process for preparing a porphyrin amino acid adduct which comprises reacting an amino dicarboxylic acid with a tetrapyrrole containing at least one carboxy group in a suitable solvent to form a compound of the structure:



wherein Z is the aminodicarboxylic acid residue less the amino group and X is the tetrapyrrole residue less the carboxy group and "n" is an integer from 1 to 4 inclusive, and optionally converting the product to a salt thereof.

2. The process according to Claim 1 wherein the amino acid is an alpha aminodicarboxylic acid.

3. The process according to Claim 1 wherein the tetrapyrrole has the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-OH\\-CH_3;\end{cases}$

R_2 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CH}CH_3$, acetyl, $\begin{cases}-H\\-ethyl,\end{cases}$ $-\overset{\overset{H}{|}}{C}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-CH_3\\-OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{\underset{OH,}{|}}{CH}CH_3$ $CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H\\-ethyl;\end{cases}$

R_5 is methyl;

R_6 is H, $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$ or CO_2H;

R_7 is $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$, or $\begin{cases}-CH_2CH_2CO_2H\\-H;\end{cases}$

R_8 is methyl or $\begin{cases}-CH_3\\-H\end{cases}$

R_9 is H, COOH, CH_2COOH or methyl;

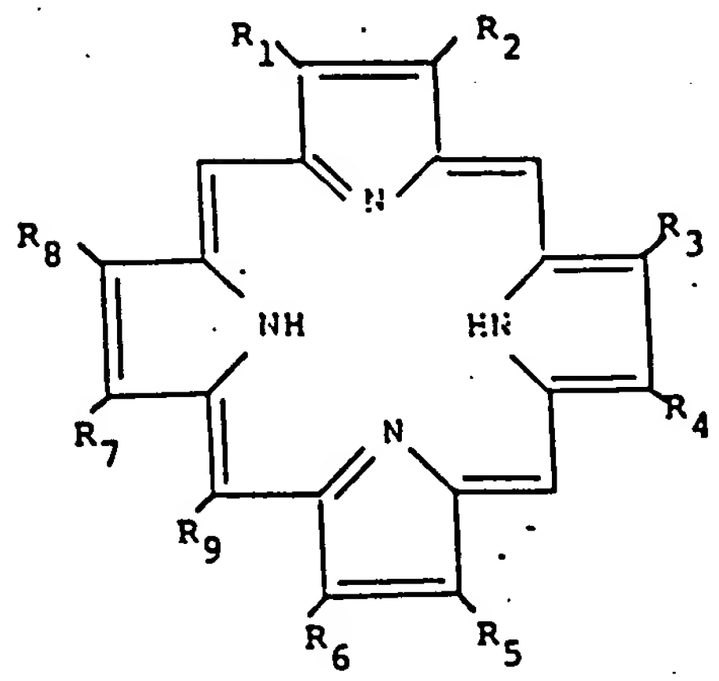
provided that when R_1, R_2, R_3, R_4, R_7 and R_8 represent two substituents or are divalent and attached to the same carbon, the respective pyrrole ring to which attached is a dihydropyrrole;

R is lower alkyl or benzyl; $-\overset{\overset{O}{\|}}{C}=O$ $-\overset{\overset{O}{\|}}{C}=O$

R_6 and R_9, taken together are $-CH_2$ or $-\underset{\underset{CO_2CH_3}{|}}{CH}$

with the proviso that at least one of R_1 - R_9 includes a free carboxyl group; and optionally converting the product to a salt thereof.

4. The process according to Claim 1 wherein the tetrapyrrole has the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H \\ -CH_3\end{cases}$ or $\begin{cases}-OH \\ -CH_3;\end{cases}$

R_2 is H, vinyl, ethyl, $-\underset{OH}{CHCH_3}$, acetyl, $\begin{cases}-H \\ -ethyl,\end{cases}$ $-\overset{H}{\underset{|}{C}}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H \\ -CH_3\end{cases}$ or $\begin{cases}-CH_3 \\ -OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{OH}{CHCH_3}$, $CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H \\ -ethyl;\end{cases}$

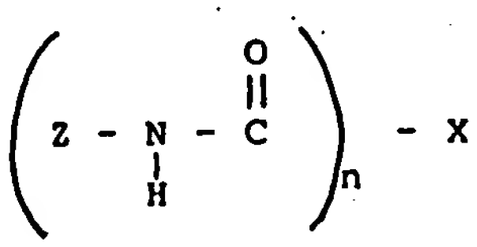
5. The process according to Claim 4 wherein the tetrapyrrole is a porphyrin, a chlorin or a bacteriochlorin.

6. The process according to Claim 4 wherein the amino acid is an alpha aminodicarboxylic acid, an aspartic acid or a glutamic acid.

7. The process according to Claim 1 wherein the porphyrin amino acid adduct is selected from the group monoaspartyl transmesochlorin IX, diaspartyl transmesochlorin IX, monoglutamyl transmesochlorin IX, diglutamyl transmesochlorin IX, monoaspartyl chlorin $\underline{e_6}$, triaspartyl chlorin $\underline{e_6}$, monoglutamyl chlorin $\underline{e_6}$, diglutamyl protoporphyrin IX, monoaspartyl mesochlorin $\underline{e_6}$, monoglutamyl protoporphyrin IX, monoaspartyl mesoporphyrin IX, diaspartyl mesoporphyrin IX, diglutamyl mesoporphyrin IX, diaspartyl protoporphyrin IX, monoaspartylbacteriochlorin $\underline{e_4}$, diaspartyl deuteroporphyrin IX, monoaspartyl deuteroporphyrin IX, monoglutamylbacteriochlorin $\underline{e_4}$, diglutamyl deuteroporphyrin IX, mono- or diaspartyl photoprotoporphyrin IX, mono- or diglutamyl photoprotoporphyrin IX, mono-, di-, tri- or tetraglutamyl coporphyrin III, mono- or diaspartyl hematoporphyrin IX,

mono- or diglutamyl hematoporphyrin IX, mono- or diglutamyl chlorin e_4, mono- or diglutamyl mesochlorin e_4, mono- or diaspartyl chlorin e_4 and monoglutamyl deuteroporphyrin IX.

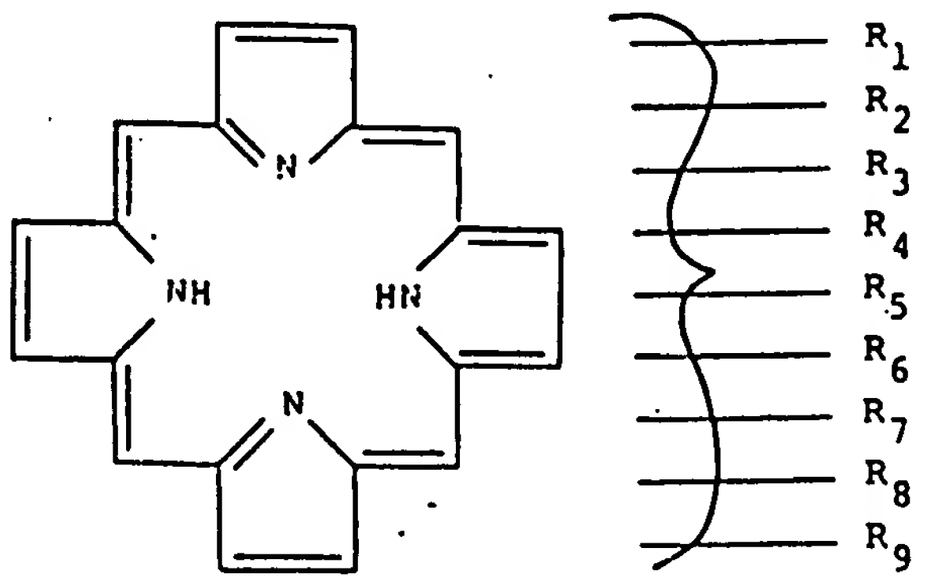
8. A therapeutic composition for detection and/or treatment of mammalian tumors which comprises a fluorescent mono- or polyamide of an aminodicarboxylic acid and a tetrapyrrole containing at least one carboxy group of the structure:



wherein Z is the aminodicarboxylic acid residue less the amino group and X is the tetrapyrrole residue less the carboxy group and "n" is an integer from 1 to 4 inclusive, and a pharmaceutical carrier therefor.

9. The therapeutic composition according to Claim 1 wherein the amino acid is an alpha aminodicarboxylic acid.

10. A therapeutic composition according to claim 8 which comprises a fluorescent mono- or polyamide of an aminodicarboxylic acid and a tetrapyrrole compound of the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-OH\\-CH_3\end{cases}$;

R_2 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CHCH_3}$, acetyl, $\begin{cases}-H\\-ethyl,\end{cases}$

$-\overset{\overset{H}{|}}{C}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-CH_3\\-OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CHCH_3}$,

$CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H\\-ethyl;\end{cases}$

R_5 is methyl;

R_6 is H, $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$ or CO_2H;

R_7 is $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$, or $\begin{cases}-CH_2CH_2CO_2H\\-H;\end{cases}$

R_8 is methyl or $\begin{cases}-CH_3\\-H\end{cases}$

R_9 is H, COOH, CH_2COOH or methyl;

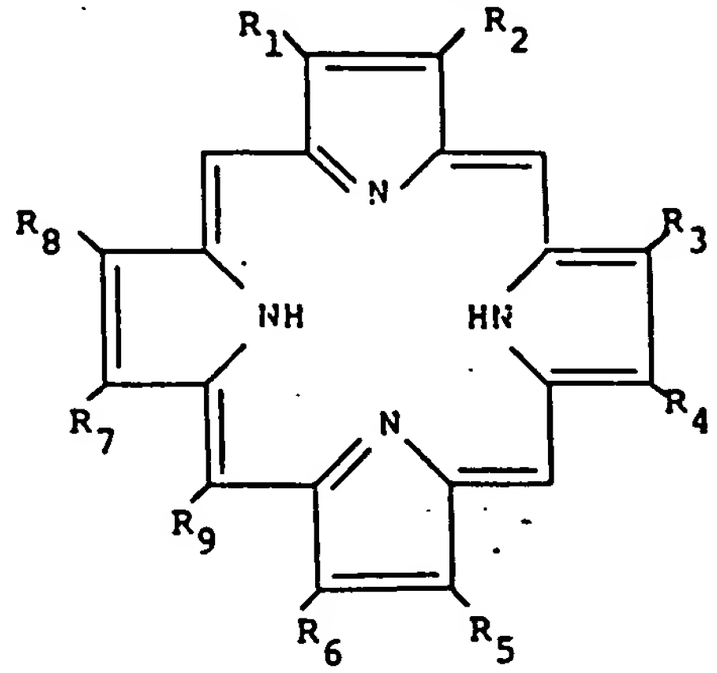
provided that when R_1, R_2, R_3, R_4, R_7 and R_8 represent two substituents or are divalent and attached to the same carbon, the respective pyrrole ring to which attached is a dihydropyrrole;

R is lower alkyl or benzyl; $-\underset{\underset{CH_2}{|}}{C}=O$ $-\underset{\underset{CHCO_2CH_3}{|}}{C}=O$

R_6 and R_9, taken together are or

with the proviso that at least one of R_1-R_9 includes a free carboxyl group; and salts thereof, and a pharmaceutically acceptable carrier therefor.

11. A therapeutic composition according to claim 8 which comprises a fluorescent mono- or polyamide of an aminodicarboxylic acid and fluorescent tetrapyrrole compound of the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-OH\\-CH_3\end{cases}$;

R_2 is H, vinyl, ethyl, $-\underset{OH}{CHCH_3}$, acetyl, $\begin{cases}-H\\-ethyl,\end{cases}$

$-\overset{H}{\underset{|}{C}}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-CH_3\\-OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{OH}{CHCH_3}$,

$CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H\\-ethyl;\end{cases}$

R_5 is methyl;

R_6 is H, $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$ or CO_2H;

R_7 is $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$, or $\begin{cases} -CH_2CH_2CO_2H \\ -H \end{cases}$;

R_8 is methyl or $\begin{cases} -CH_3 \\ -H \end{cases}$;

R_9 is H, COOH, CH_2COOH or methyl;

provided that when R_1, R_2, R_3, R_4, R_7 and R_8 represent two substituents or are divalent and attached to the same carbon, the respective pyrrole ring to which attached is a dihydropyrrole;

R is lower alkyl or benzyl;

R_6 and R_9, taken together are $-\overset{\displaystyle -C=O}{\underset{\displaystyle |}{CH_2}}$ or $-\overset{\displaystyle -C=O}{\underset{\displaystyle |}{CHCO_2CH_3}}$

with the proviso that at least one of R_1-R_9 includes a free carboxyl group; and salts thereof, and a pharmaceutically acceptable carrier therefor.

12. The therapeutic composition according to Claim 11 wherein the tetrapyrrole is a porphyrin, a chlorin or a bacteriochlorin.

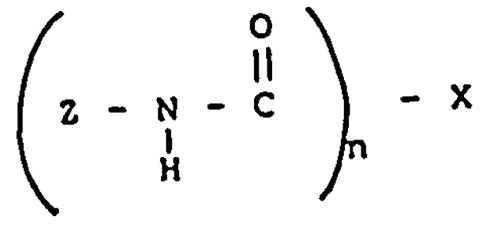
13. The therapeutic composition according to Claim 7 wherein the amino acid is an alpha aminodicarboxylic acid, an aspartic acid or an glutamic acid.

14. The therapeutic composition according to Claim 11 wherein the amide is selected from the group of Claim 7.

15. Use of a compound as described in Claims 8 to 14 for the preparation of a therapeutic composition for detecting and/or treatment of mammalian tumors.

CLAIMS

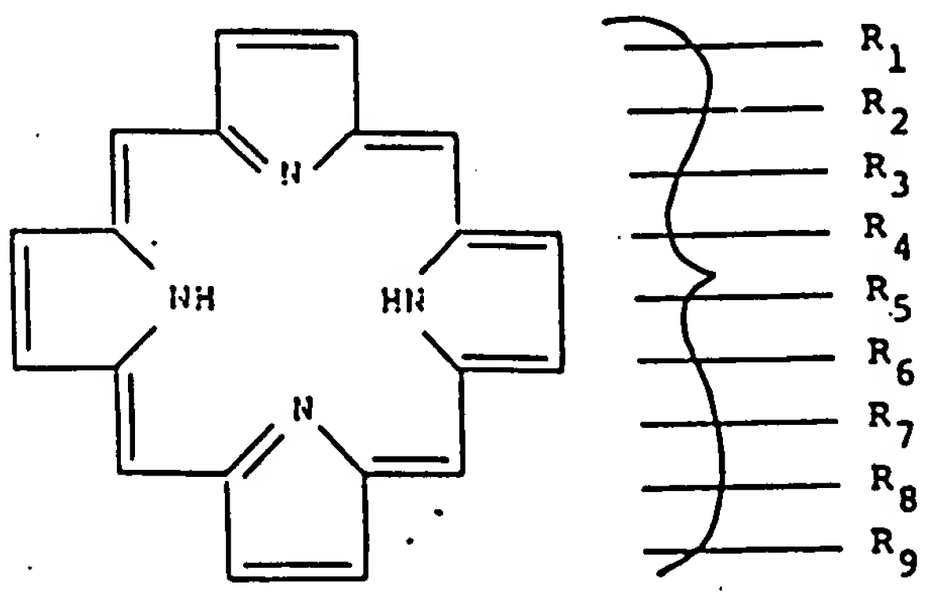
1. A process for preparing a porphyrin amino acid adduct which comprises reacting an amino dicarboxylic acid with a tetrapyrrole containing at least one carboxy group in a suitable solvent to form a compound of the structure:



wherein Z is the aminodicarboxylic acid residue less the amino group and X is the tetrapyrrole residue less the carboxy group and "n" is an integer from 1 to 4 inclusive, and optionally converting the product to a salt thereof.

2. The process according to Claim 1 wherein the amino acid is an alpha aminodicarboxylic acid.

3. The process according to Claim 1 wherein the tetrapyrrole has the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-OH\\-CH_3;\end{cases}$

R_2 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CH}CH_3$, acetyl, $\begin{cases}-H\\-ethyl,\end{cases}$ $-\overset{\overset{H}{|}}{C}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H\\-CH_3\end{cases}$ or $\begin{cases}-CH_3;\\-OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{\underset{OH}{|}}{CH}CH_3$, $CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H\\-ethyl;\end{cases}$

R_5 is methyl;

R_6 is H, $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$ or CO_2H;

R_7 is $CH_2CH_2CO_2H$, $CH_2CH_2CO_2R$, or $\begin{cases}-CH_2CH_2CO_2H\\-H;\end{cases}$

R_8 is methyl or $\begin{cases}-CH_3\\-H\end{cases}$

R_9 is H, COOH, CH_2COOH or methyl;

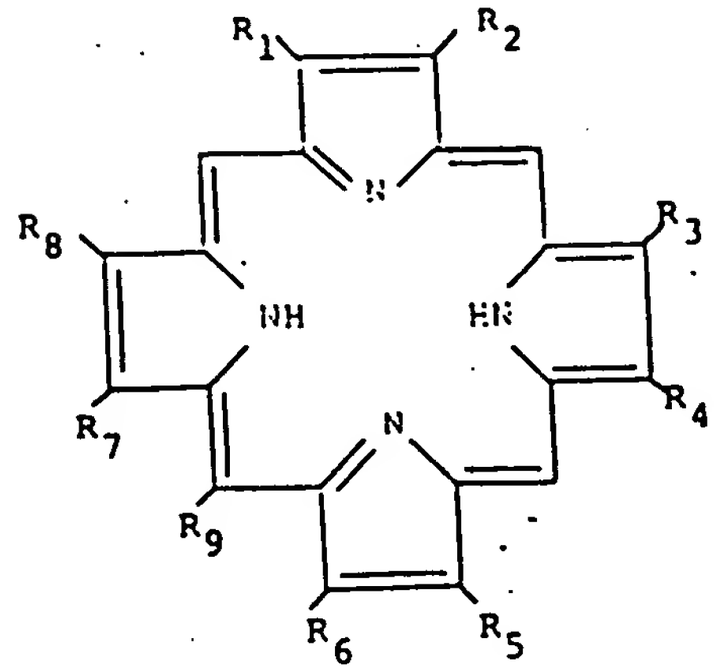
provided that when R_1, R_2, R_3, R_4, R_7 and R_8 represent two substituents or are divalent and attached to the same carbon, the respective pyrrole ring to which attached is a dihydropyrrole;

R is lower alkyl or benzyl; $-\overset{\overset{O}{\|}}{C}=O$ $-\overset{\overset{O}{\|}}{C}=O$

R_6 and R_9, taken together are $-CH_2$ or $-\underset{\underset{CH_2CO_2CH_3}{|}}{CH}$

with the proviso that at least one of R_1 - R_9 includes a free carboxyl group; and optionally converting the product to a salt thereof.

4. The process according to Claim 1 wherein the tetrapyrrole has the formula:



or the corresponding di- or tetrahydrotetrapyrroles wherein

R_1 is methyl; $\begin{cases}-H \\ -CH_3\end{cases}$ or $\begin{cases}-OH \\ -CH_3;\end{cases}$

R_2 is H, vinyl, ethyl, $-CHCH_3$ (OH), acetyl, $\begin{cases}-H \\ -ethyl,\end{cases}$ $-\overset{H}{\underset{|}{C}}=O$, $CH_2CH_2CO_2H$, or =CHCHO;

R_3 is methyl $\begin{cases}-H \\ -CH_3\end{cases}$ or $\begin{cases}-CH_3 \\ -OH;\end{cases}$

R_4 is H, vinyl, ethyl, $-\underset{OH}{\underset{|}{C}}HCH_3$, $CH_2CH_2CO_2H$, =CHCHO; or $\begin{cases}-H \\ -ethyl;\end{cases}$

5. The process according to Claim 4 wherein the tetrapyrrole is a porphyrin, a chlorin or a bacteriochlorin.

6. The process according to Claim 4 wherein the amino acid is an alpha aminodicarboxylic acid, an aspartic acid or a glutamic acid.

7. The process according to Claim 1 wherein the porphyrin amino acid adduct is selected from the group monoaspartyl transmesochlorin IX, diaspartyl transmesochlorin IX, monoglutamyl transmesochlorin IX, diglutamyl transmesochlorin IX, monoaspartyl chlorin $\underline{e_6}$, triaspartyl chlorin $\underline{e_6}$, monoglutamyl chlorin $\underline{e_6}$, diglutamyl protoporphyrin IX, monoaspartyl mesochlorin $\underline{e_6}$, monoglutamyl protoporphyrin IX, monoaspartyl mesoporphyrin IX, diaspartyl mesoporphyrin IX, diglutamyl mesoporphyrin IX, diaspartyl protoporphyrin IX, monoaspartylbacteriochlorin $\underline{e_4}$, diaspartyl deuteroporphyrin IX, monoaspartyl deuteroporphyrin IX, monoglutamylbacteriochlorin $\underline{e_4}$, diglutamyl deuteroporphyrin IX, mono- or diaspartyl photoprotoporphyrin IX, mono- or diglutamyl photoprotoporphyrin IX, mono-, di-, tri- or tetraglutamyl coporphyrin III, mono- or diaspartyl hematoporphyrin IX,

mono- or diglutamyl hematoporphyrin IX, mono- or diglutamyl chlorin e_4, mono- or diglutamyl mesochlorin e_4, mono- or diaspartyl chlorin e_4 and monoglutamyl deuteroporphyrin IX.

8. Use of a compound prepared by a process according to Claims 1 to 7 for the preparation of a therapeutic composition for detecting and/or treatment of mammalian tumors.